

ISOLATION OF CYTOPATHIC PORCINE ROTAVIRUSES IN CELL CULTURES
AND THEIR SEROTYPING BY SERUM NEUTRALIZATION TESTS

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[Introduction]

Rotaviruses are difficult to grow in cell cultures, and so far only rotaviruses from calves have been serially passaged in cell cultures.

Porcine rotavirus could be serially passaged in primary porcine kidney cell cultures when the virus inoculum was treated with pancreatin or trypsin before inoculation to the cell cultures (Theil et al, 1977), although, a clear cytopathic effect was not observed in the infected cells. Recently, we reported that high titer producing porcine rotavirus showing clear cytopathic effect was consistently isolated in roller cultures of MA-104 cells by a combination of trypsin treatment of the inoculum and incorporation of trypsin in the maintenance medium (Fukusho et al, 1981).

On the other hand, it was reported that there are at least 2 to 4 distinct serotypes among human rotaviruses. However, there are no report on antigenic differences among porcine rotaviruses.

This paper describes isolation of the cytopathic porcine rotaviruses in cell cultures from feces of piglets with diarrhea of field cases. Serotypes of the isolated porcine rotaviruses were investigated by serum neutralization tests.

[Methods]

The fecal samples were obtained from piglets with diarrhea in a pig farms and used for virus isolation. Prior to inoculation, the fecal samples were treated with 10 µg of crystalline trypsin per ml for 30 minutes at 37°C. Trypsin treated samples were inoculated onto confluent sheets of MA-104 cells in tubes and allowed to absorb at 37°C. After 1 hour, the inoculum was removed and monolayers were washed once with 5 ml of phosphate buffered saline. The cultures were refed with an Eagle's MEM with 0.5 µg of crystalline trypsin per ml. The infected cells were incubated 2 to 3 days in a roller apparatus at 37°C and allowed next passage with above mentioned methods. Neutralization tests were done by the methods described before (Fukusho et al, 1981). Antisera against porcine rotavirus strains were obtained by immunizing guinea pigs which had been free from rotavirus antibody by complement fixation tests. RNA-PAGE was done by the methods of Kalica et al (1978).

[Results]

Isolation of cytopathic porcine rotaviruses from feces of piglets with diarrhea of field cases : Of 72 samples of feces examined for rotavirus, 24 were positive by fluorescent antibody staining. These rotavirus positive samples were used for virus isolation. Eighteen cytopathic porcine rotaviruses were isolated in roller cultures of the MA-104 cells by serial passages of virus in the intervals every 2 to 3 days. The cytopathic effect was clearly observed after 8 to 10 serial passages and virus titer at 10 to 18 passage levels was 10^7 TCID₅₀ per ml or higher. The isolated porcine rotaviruses agglutinated erythrocytes of pigs, guinea pigs and human.

Differentiation of porcine rotaviruses by serum neutralization tests and RNA-PAGE : Cross neutralization tests were done to determine serotypes among 16 strains of cytopathic porcine rotaviruses isolated by the above

methods. Of the strains, 3 were well neutralized only by homologous antiserum and 13 were neutralized strongly by not only respective homologous antiserum but also some of the heterologous antisera. As the results, they were classified antigenically into 7 serotypes. Additional 3 isolates were tested by one-way cross neutralization tests using antisera against the above 7 serotype strains. They were poorly neutralized by any of the antisera. These results showed that there might be more than 10 serotypes.

The viral RNA of different serotypes of porcine rotaviruses was separated by PAGE. Differences in RNA migration pattern were not observed distinctly among them, although, some strains showed slight differences in the 4th segment of the 1st group of the viral RNA. RNA migration pattern of porcine rotaviruses was different from bovine and human rotaviruses. The 2s type of RNA migration pattern (Espejo et al, 1979) was not detected in porcine rotaviruses.

[Conclusions]

Our results confirmed that the methods reported by Fukusho et al (1981) is very useful for isolation of cytopathic porcine rotaviruses from feces of piglets with diarrhea of field cases.

By cross neutralization tests, we presumed that there are at least 10 serotypes among porcine rotaviruses. It seems that some viral strains possessing antigen with other strains may be variants produced from the distinct serotype strain having only own antigen.

On the other hand, the RNA migration pattern is not useful tool for studying the differences among porcine rotaviruses.

[Selected references]

- Espejo, R.T. et al. : J. Infect. Dis. 139, 474-477, 1979.
Kalica, A.R. et al. : Virol. 87, 247-255, 1978.
Fukusho, A. et al. : Arch. Virol. 69, 49-60, 1981.